

Nestlings' carotenoid feather ornament affects parental allocation strategy and reduces maternal survival

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Abstract

In some birds, feather ornaments are expressed in nestlings well before sexual maturation, possibly in response to parental favouritism towards high-quality offspring. In species with synchronous hatching, in which nestling ornaments may vary more among than within broods, parents may use this information to adjust their parental allocation to the current brood accordingly. We tested this hypothesis in the rock sparrow, in which a sexually selected yellow feather ornament is also expressed in nestlings. We experimentally enlarged nestlings' breast patch in a group of broods and sham-manipulated another group of control broods. Nestlings with enlarged ornament were fed more frequently and defended more actively from a dummy predator than their control counterparts. Mothers from the enlarged group were more likely to lay a second clutch and showed a reduced survival to the next breeding season. These results provide one of the first evidences of differential parental allocation among different broods based directly on nestlings' ornamentation, and the first, to our knowledge, to show a reduction in maternal survival.

Introduction

Ornaments based on elaborated feather colours are widespread in birds. Confirming Darwin's hypothesis (Darwin, 1859), a large number of studies have demonstrated that these ornamental traits have evolved in response to a preference for mates with more colourful plumage ornaments (see Hill & McGraw, 2006 for a recent review). Fascinatingly, such ornamental traits are sometimes also expressed in the young of some species (Krebs & Putland, 2004; Tschirren *et al.*, 2005; Bize *et al.*, 2006a; Galván & Alonso-Alvarez, 2008). Clearly, ornamentation in nestlings cannot be favoured by sexual selection and calls for alternative explanations.

One alternative explanation is that nestling ornamentation may be the result of a genetic correlation with signal expression later in life. If signal production does not have a large cost, then turning the genetic machinery controlling its expression during development off in early life and on again later may not evolve. This nonadaptive explanation may account for those cases in which offspring ornamentation is a similar version of adult ornaments. For example, in the great tit, *Parus major*, yellow feathers of nestlings' breast do not have any apparent function in parent-offspring signalling (Tschirren *et al.*, 2005; but see Fitze & Tschirren, 2006; Tanner & Richner, 2008).

Alternatively, it has been hypothesized that parents use nestling ornamentation to adjust resource distribution among offspring, as they should have been selected to provide care in relation to the reproductive value of the offspring (Clutton-Brock, 1991). This will maximize parents' lifetime reproductive success if offspring survival and future reproductive success are higher in

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high-quality nestlings. Indeed, both models of resolution of parental–offspring conflict (Godfray, 1991, 1995) and empirical studies (Lyon *et al.*, 1994; Saino *et al.*, 2000; Bize *et al.*, 2006b; de Ayala *et al.*, 2007) have shown that the evolution of costly signals in offspring can be promoted by parental favouritism when nest-mates compete for the limited resources delivered by parents. In precocial birds, the young express specific ornaments only during the period of intense parental care, and this suggests that young ornamentation has evolved to signal offspring quality to parents (Krebs & Putland, 2004). Indeed, in their classical study, Lyon *et al.* (1994) experimentally trimmed in some nestlings the red filoplumes that ornament nestlings' head in the American coot, *Fulica americana*, and found that they had a reduced body growth and survival rate in comparison with their fully ornamented nest-mates. In synchronous hatching birds, however, nestling ornamentation may vary not only within brood but also among broods (see below). One may therefore predict that nestling ornamentation also affects parental allocation among subsequent broods, trading off current reproduction with future reproduction or survival, a possibility that has never been explored so far.

To explore these hypotheses, we investigated the role of nestling ornamentation in parental care in the rock sparrow, *Petronia petronia*. In this passerine, adults of both sexes possess a yellow, carotenoid-based breast patch that is subject to mutual mate choice (Pilastro *et al.*, 2003; Griggio *et al.*, 2005a) and to intra-sexual selection (male–male competition, Griggio *et al.*, 2007). A reduced version of the adult breast patch, however, is present also in nestlings; they have a small yellow breast patch whose size, at fledging, can vary considerably among different broods. In particular, the variance in this ornament size is larger between broods than within broods. Yellow breast patch size seems to be an indicator of nestling immunological competence. In a parallel study the difference between the wing-web thickness before and after phytohaemagglutinin (PHA) injection was used as an assessment of the cell-mediated immune response (Smits *et al.*, 1999). It was observed that yellow breast patch size is positively associated with the T-cell-mediated immune response of nestlings (M. Griggio, unpublished results).

The majority of the studies conducted so far on the role of nestling ornamentation have been based on the experimental manipulation of the signal in some of the nestlings within a brood and the comparison of the feeding rate between manipulated and control brood mates (e.g. Saino *et al.*, 2000; Bize *et al.*, 2006b). This approach emphasizes the evolution of nestling ornamentation in relation to parental favouritism and intra-brood competition. To test the possibility that parents are influenced by the expression of nestling ornamentation to adjust their parental allocation to the current reproductive episode, we adopted an experimental design in

which the yellow breast patch of all nestlings in a randomly selected group of broods was enlarged, whereas the ornament of control group of broods was sham-manipulated (Lyon *et al.*, 1994). We then compared the number of feeding trips and the size of the prey before and after manipulation in the two groups. Furthermore, we simulated an attack by a predator using a mounted weasel, *Mustela nivalis*, to test whether brood defence was influenced by the manipulation of the size of nestlings' ornament. To evaluate the effect of parental allocation into current reproduction on future reproduction and survival, we determined the proportion of females that laid a second clutch within the same breeding season and adult and offspring survival to the next breeding season. We predicted that broods in which nestlings' yellow patch was experimentally enlarged would be fed and defended against predators by their parents more intensely and would show an increased survival rate of nestlings, as compared with their control counterparts. In contrast, we expected that the increased parental effort would reduce future reproduction and survival of the adults.

Materials and methods

Study species

The rock sparrow is a monomorphic cavity-nesting passerine (Cramp & Perrins, 1994) characterized by a yellow breast patch of roughly trapezoid shape (Pilastro *et al.*, 2003). Nonbreeding individuals have smaller yellow patches than both breeding males and females, and in females the yellow patch size is correlated with body mass (Pilastro *et al.*, 2003). This breast patch is also present, albeit slightly reduced in size compared with adults, in nestlings, in which it becomes visible usually from an age of fourteen days (nestlings remain in the nest for about eighteen days, Griggio *et al.*, 2003). Reflectance spectra of the nestlings yellow breast patch are similar to that of adults (M. Griggio and A. Pilastro, unpublished observations; see also Serra *et al.*, 2007). The size of the yellow breast patch at this age is smaller in nestlings (mean width: 7.37 mm \pm 2.86 SD, range: 0–17.5 mm, $n = 243$) than that of the adults (see the section 'Nestling breast patch manipulation'), and shows a large variation among broods (45.0% of the total variance in patch size, estimated with SPSS 15.0, VARCOMP procedure, brood identity as random factor; number of broods = 44, number of nestling = 243). Moreover, nestlings' yellow patch size is positively correlated with their body mass (Pearson correlation: $r = 0.20$, $P = 0.001$, number of nestling = 243), and the increased body size might reflect higher offspring quality (e.g. better survival) as observed in other passerine species (e.g. Naef-Daenzer *et al.*, 2001).

The population in our study area presents a wide array of mating patterns from monogamy to polygamy (Pilastro

et al., 2001, 2002). In this study only two males were polygamous, but including or excluding these four nests from the analysis did not substantially change the results (see below). Moreover, although both parents cooperate in feeding their chicks, most of the feeding effort is usually carried out by the female (Pilastro *et al.*, 2001; Griggio & Pilastro, 2007). In contrast, in the case of simulated predator attacks, both parents actively defend the nestlings by directly mobbing and attacking the dummy predator (Griggio *et al.*, 2003). Therefore in these cases analyses were based only on those nests in which both parents were present.

Study area and field methods

The study was carried out during two breeding seasons (2004 and 2005), between May and August, in West Alps, in Val de la Clarée (Briançon), France. In this area, 43 nest boxes were set up in two neighbouring villages in 2000 (Matessi *et al.*, 2005); field methods are described in detail in Pilastro *et al.* (2003) and Griggio *et al.* (2003, 2005b). Briefly, nest boxes were checked twice a week to determine pair bonds, laying date, clutch size, hatching and fledging date. The body mass of adults and chicks was measured to the nearest 0.1 g. Breast patch size of adults was measured by placing a transparent acetate strip over the breast, drawing the contour of the breast patch onto the strip and subsequently measuring the major, horizontal axis of the outline with a calliper to the nearest 0.1 mm. Patch size varies mainly in width and this measure in adult birds has a high repeatability (Pilastro *et al.*, 2002; Griggio *et al.*, 2003).

Nestling breast patch manipulation

We randomly assigned 49 broods to two experimental groups, controls and enlarged breast patch. Nestlings of 25 broods (nine in 2004 and 16 in 2005) were manipulated at an age of 14 days; nestlings of 24 control broods were manipulated as in the enlarged group except for patch enlargement (10 in 2004 and 14 in 2005). To enlarge the breast patch we painted the beige feathers surrounding the yellow ornament with picric acid diluted in ethanol, following an established procedure (for more details see Pilastro *et al.*, 2003 and Griggio *et al.*, 2007). This method modified the reflectance spectra of the plumage similarly to the spectra of natural yellow breast patch of nestlings (M. Griggio and A. Pilastro, unpublished observations). Control broods underwent the same manipulation except that we applied only water and ethanol around the breast patch without modifying its size. During each breeding season we randomly assigned the first brood to one of the two groups and subsequently alternated the treatment; this allowed us to distribute early and late broods equally to the two experimental groups.

For both treatments we measured the breast patch size before and after manipulation (for more details see

above). We calculated repeatability (Lessells & Boag, 1987) and its SE (Becker, 1984) from double measurements of 44 breast patches (26 pretreatment and 18 after-treatment patches). Repeatability was equal to 0.925 (± 0.022 SE; $F_{43,87} = 25.84$, $P < 0.001$).

Neither nestling mean body mass (control: $31.38 \text{ g} \pm 1.94$ SD; enlarged: $31.50 \text{ g} \pm 1.23$ SD; year, $F_{1,46} = 0.05$, $P = 0.82$; treatment, $F_{1,46} = 0.07$, $P = 0.80$) nor mean nestling breast patch size before manipulation (control: $7.48 \text{ mm} \pm 2.63$ SD; enlarged: $7.06 \text{ mm} \pm 2.29$ SD; year, $F_{1,41} = 0.236$, $P = 0.63$; treatment, $F_{1,41} = 0.279$, $P = 0.60$) differed significantly between experimental groups. Considering the breast patch measurements of the same individual nestlings before and after manipulation, breast patch size in the enlarged group changed from $7.27 \text{ mm} \pm 2.99$ SD to $12.36 \text{ mm} \pm 2.68$ SD (paired t test, $t_{134} = 15.15$, $P < 0.001$). In contrast, sham manipulation of the breast patch in the control group did not significantly affect its size (before: $7.30 \text{ mm} \pm 2.43$ SD; after: $7.25 \text{ mm} \pm 2.21$ SD ($t_{84} = 0.317$, $P = 0.75$). Mean enlargement of the breast patch size was within the range of variation observed in unmanipulated nestlings (0–17.5 mm), apart from four nestlings (of 145 measured) whose breast patch after manipulation slightly exceeded the maximum unmanipulated size (similar results were obtained when we excluded these four nestlings from our analyses).

There were no differences between treatment groups in parental breast patch size (male: control group 15.4 ± 0.52 mm; treatment group 14.75 ± 0.81 mm; $t_{24} = 0.676$, $P = 0.50$; female: control group 12.68 ± 0.5 mm; treatment group 12.11 ± 0.38 mm; $t_{30} = 0.9$, $P = 0.37$) and in parental body mass (male: control group 33.11 ± 0.52 g; treatment group 32.43 ± 0.61 g; $t_{33} = 0.84$, $P = 0.41$; female: control group 32.31 ± 0.31 g; treatment group 32.04 ± 0.63 g; $t_{33} = 0.39$, $P = 0.70$). There were no differences in the date of first egg laid and brood size between the two study years and between treatment groups (year: laying date: $F_{1,44} = 0.43$, $P = 0.51$; brood size: $F_{1,44} = 0.20$, $P = 0.67$; treatment: laying date: $F_{1,44} = 0.27$, $P = 0.60$; brood size; $F_{1,44} = 0.19$, $P = 0.66$, two-way ANOVA).

Sample size can vary between analyses since when weather conditions were unfavourable we limited brood manipulation time and in four cases we did not measure pretreatment nestlings' breast patch.

Parents' feeding rate

We observed both treatment and control nests using 20–60 × spotting scopes about 50 m from the nest for approximately 1.5 h on two consecutive days before and two consecutive days after the day of treatment, for an average 6.2 h of observation per nest (SD = 0.79, range = 4.5–8 h). In total, in the two study years, we collected 151 h of observations for controls and 153 h for treatment groups.

For each parent we recorded the number of feeding trips and prey size. We estimated prey item size (mainly grasshoppers and caterpillars) in three categories by comparing it with adult bill length (0.5 bills, 1 bill, 2 bills or larger), according to Griggio & Pilastro (2007). Feeding rate was then calculated as the number of feeding trips times the mean prey size.

Parents' nest defence

After the last hour of observation (i.e. when nestlings were 17-days old) we tested the intensity of nest defence against a terrestrial nest-predator in 24 pairs (12 controls and 12 enlarged). We ran the defence test between 6:00 and 8:00 a.m., by placing a mounted weasel on top of the nest box (see Griggio *et al.*, 2003). Once the observer was at 25–30 m away, a 15-min maximum latency interval started; if in this interval at least one of the members of the pair arrived, the experiment began and the behaviour of the individual(s) was recorded for 10 min, after which the predator was removed and the experiment ended. Therefore the maximum total treatment time (and disturbance to the nest) was 25 min. We measured for each parent: latency to arrive at the nest; the number of attacks min^{-1} against the predator (direct flight towards the predator); the number of alarm calls min^{-1} and the total time spent within 15 m of the predator (proportion of time).

Estimate of survival rate

All adults and nestlings were ringed with a numbered aluminium ring and with a unique combination of colour rings that allowed their identification from a distance. We considered as locally recruited those offspring that were observed at least once in the natal area in the following breeding season (2005 and 2006), according to the same methodological procedure used in Tavecchia *et al.* (2002). We can therefore assume that the 'recapture' effort in the two years was comparable.

Statistical methods

The feeding rate of the parents in the two groups was compared using a GLM model. To standardize the feeding effort among different nests we used the analysis of covariance in which the feeding rate after manipulation was the dependent variable and the brood size (or the feeding rate before manipulation) was the covariate. Experimental group and year were entered as fixed factors. The Levene test for homogeneity of variance was used (all $P > 0.06$). The analyses using brood size and those using the feeding rate before manipulation as covariates gave similar results and the results relative to the models with brood size as covariate are given as supporting information (Table S1). Defence rate against a predator dummy was analysed with a mixed linear model

in which we entered latency, number of attacks min^{-1} and number of alarm calls min^{-1} as the dependent variable, nest identity as random factor and treatment and sex of the parent as fixed factors. This allowed us to simultaneously test the defence rate of males and females, statistically controlling for the fact that the defence rate of the two parents was significantly correlated (see below). Multivariate normality and homogeneity of variance-covariance matrices were tested by Box's M test ($P = 0.09$). Statistical tests were performed with SPSS v.15. If not otherwise stated, mean \pm SE is presented.

Results

Parents' feeding behaviour

Parents of the two groups did not differ in their nestling feeding rate before manipulation (treatment, $F = 0.09$, $P = 0.77$; brood size (covariate), $F_{1,46} = 19.23$, $P < 0.001$, interaction treatment \times brood size, $P = 0.79$; Fig. 1a). In contrast, parents of the enlarged group fed their nestling at a significantly higher rate as compared to their control counterparts (treatment, $F_{1,46} = 14.27$, $P < 0.0001$; brood size (covariate), $F_{1,46} = 35.55$, $P < 0.0001$, interaction treatment \times brood size, $P = 0.43$; Fig. 1b). This increase was mainly due to the female, as only six males were observed feeding their brood before manipulation and five after manipulation. Among these latter, three males (all controls) were paired with females that deserted their brood and they took over all the feeding care after female desertion.

Parents' defence behaviour

In one case (a control nest) none of the parents arrived within 15 min and the experiment was terminated before any observation was made. In the remaining 23 nests, all females were observed at the nest within

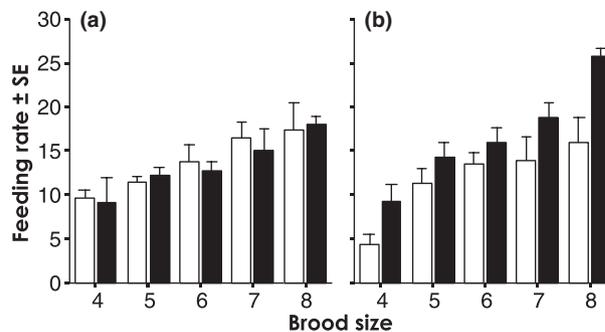


Fig. 1 Mean (\pm SE) feeding rate (number of trips $\text{h}^{-1} \times$ mean prey size) in relation to nestling breast patch size manipulation and brood size (control: white bars; enlarged: black bars). Before manipulation (a) and after manipulation (b).

15 min from the beginning of the experiment, whereas the male was not observed in six cases. In the 17 nests from which we could observe the defence behaviour of both parents (eight controls and nine enlarged), all three measures of brood defence were highly correlated between male and female (latency: $r_{16} = 0.75$, $P < 0.001$; number of attacks min^{-1} : $r_{16} = 0.79$, $P < 0.001$; number of alarm calls min^{-1} : $r_{16} = 0.66$, $P = 0.004$; log transformation). Overall, the three defence variables measured were significantly influenced by treatment and sex (multivariate linear mixed model, treatment, Wald $\chi^2 = .99$, d.f. = 3, $P < 0.001$; sex, Wald $\chi^2 = 28.80$, d.f. = 3, $P < 0.001$; treatment \times sex, Wald $\chi^2 = 2.25$, d.f. = 3, $P = 0.08$; nest identity entered as random factor). Univariate analyses revealed that latency did not differ between sexes and treatment groups, whereas number of attacks and frequency of alarm calls differed significantly in relation to sex and treatment, with females and parents of the enlarged patch group defending the brood more intensely than males and controls, respectively (Fig. 2, Table 1). Even considering all the nests together, the number of attacks performed by females differed significantly between treatment groups (control group: 6.09 ± 2.06 , $n = 11$, enlarged patch group: 18.08 ± 4.91 , $n = 12$; univariate analysis: $P = 0.04$). For the 23 females the other two variables measured (latency and number of alarm calls min^{-1}) were not significantly influenced by treatment (multivariate analysis: $P = 0.17$).

Female breeding strategy

In total, 18 females (13 in the enlarged group and five in the controls, $\chi^2 = 5.12$; $P = 0.024$) laid a second clutch within the same breeding season. The proportion of double brooding females was also higher in the enlarged group after we had statistically controlled for differences in laying date and year (logistic regression; treatment: Wald $\chi^2 = 5.19$; $P = 0.023$; year: Wald $\chi^2 = 0.96$, $P = 0.33$; laying date: Wald $\chi^2 = 4.58$, $P = 0.032$, interaction treatment \times laying date, $P = 0.23$; excluding the nonsignificant predictors from the model did not change the results: treatment: Wald $\chi^2 = 5.20$; $P = 0.023$; laying date: Wald $\chi^2 = 4.42$, $P = 0.036$). In particular, laying date of the first clutch was negatively associated with the

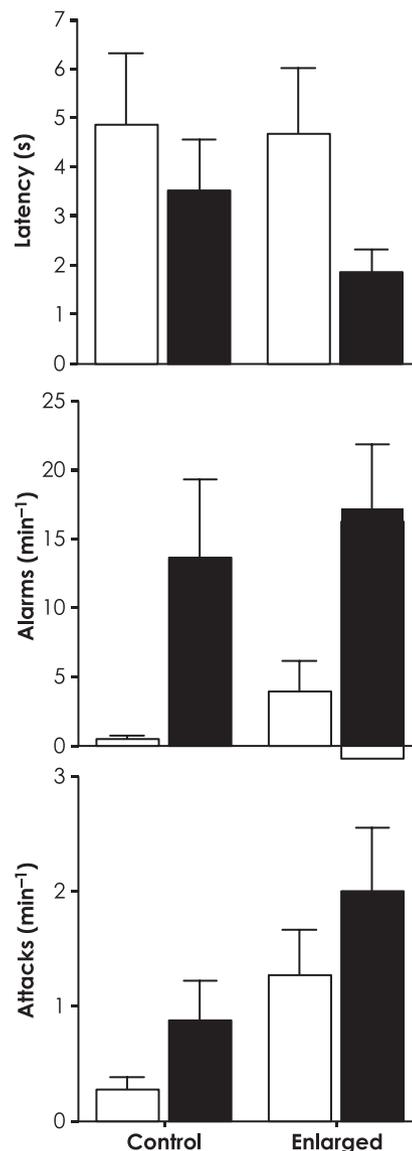


Fig. 2 Effect of nestlings ornament manipulation on the number of flight attacks per min^{-1} toward a mounted predator, performed by male (white) and female (black). Means are given \pm SE ($n = 7$ controls and $n = 8$ nests with the size of the nestlings' breast patch increased).

Table 1 Effect of nestling breast patch size manipulation on parents' defence behaviour (linear mixed model with nest as random factor)

Source	d.f.	Latency (min)		Number attacks min^{-1}		Number alarm calls min^{-1}	
		Wald χ^2	P	Wald χ^2	P	Wald χ^2	P
Treatment	1	0.20	0.65	8.64	0.003	9.13	0.003
Sex	1	0.02	0.90	11.23	<0.001	75.14	<0.0001
Treatment \times sex	1	0.22	0.64	2.88	0.09	3.65	0.056

Variables were log transformed before the analyses.

probability of laying a second clutch (laying date: Wald test, $b = -0.194 \pm 0.092$).

Adult and offspring survival

Female survival to the next breeding season was significantly lower in the enlarged group compared with its control counterpart and was positively correlated with brood size (treatment: $b = -1.268 \pm 0.4099$, Wald $\chi^2 = 9.565$, $P = 0.002$; brood size: $b = 0.715 \pm 0.1859$, Wald $\chi^2 = 14.81$, $P < 0.001$, interaction treatment \times brood size, $P = 0.12$; binomial regression analysis: dependent female survival (0 = not survived, 1 = survived); initial model included also year, lay date and interactions, which were all nonsignificant and removed from the model, Fig. 3). Male survival rate was not affected by experimental brood manipulation ($P > 0.6$).

Offspring local recruitment was not significantly affected by treatment, although there was a tendency in the expected direction. Instead, offspring local recruitment differed significantly between years and was negatively correlated with laying date (treatment: $b = -14.986 \pm 10.7470$, Wald $\chi^2 = 1.944$, $P = 0.16$; year: $b = 1.119 \pm 0.3453$, Wald $\chi^2 = 10.51$, $P = 0.001$; laying date: $b = -0.110 \pm 0.0603$, Wald $\chi^2 = 3.332$, $P = 0.068$; treatment \times laying date: $b = 0.097 \pm 0.069$, Wald $\chi^2 = 1.979$, $P = 0.16$).

Discussion

Our manipulation of a carotenoid feather signal in nestlings revealed that parents adjust their overall parental effort, both in terms of feeding rate and defence

from predators, according to the expression of nestlings' ornamentation. Moreover, mothers from the enlarged group were more likely to lay a second clutch and showed a reduced survival to the next breeding season.

Our results demonstrate that the development of a large ornament is associated with substantial benefits to the nestlings, mediated by increased parental care, and probably resulting in enhanced survival prospects. Since carotenoids used for feather coloration are metabolically lost, it may therefore be unexpected that nestlings use part of their limited carotenoid supply to produce yellow feathers that are often going to be carried for a short period, as the moult of body feathers occurs in most passerines soon after fledging (Jenni & Winkler, 1994). Because there is ample evidence of a strong link between carotenoids and immune function, parasite resistance and condition (Hudon, 1994; Lozano, 1994; Olson & Owens, 1998; Hill, 1999; Møller *et al.*, 2000; Tschirren *et al.*, 2003; Horak *et al.*, 2006; Griggio *et al.*, 2009), there should be a selection for a careful allocation of the available carotenoids between signalling and metabolic functions (Faivre *et al.*, 2003; McGraw & Ardia, 2003). In particular, because these pigments cannot be synthesized *de novo* but must be obtained from the diet, a trade-off in carotenoid allocation between maintenance and ornamentation has therefore been hypothesized (Lozano, 1994). The rationale behind this hypothesis is that individuals in better condition should require fewer carotenoids for maintenance (i.e. immune function) and therefore be able to allocate a larger portion of their limited carotenoid stores to ornamentation. This explanation is in agreement with ornaments serving as a parental signal, as found in our study.

Previous studies on the evolution of nestling ornamentation, which used an experimental design involving the manipulation of one part of the nestlings and therefore highlighted within-clutch parent favouritism found that parents do not share equally their parental effort within brood, delivering higher proportion of their food to more ornamented offspring without altering the total amount of care provided to the brood (e.g. Saino *et al.*, 2000; Bize *et al.*, 2006b). Whether or not, however, parents adjust the total amount of care allocated to a given brood (thus potentially affecting their allocation strategy between subsequent clutches) has been tested only once, and authors did not find significant differences between treatments (Lyon *et al.*, 1994).

According to the predictions of theoretical models of parent-offspring conflict (Clutton-Brock, 1991; Godfray, 1991, 1995) we found that parents increase the total amount of care given to a brood when the size of the nestlings' ornament is experimentally increased. Our results parallel those obtained in experimental tests of the differential allocation hypothesis (Burley, 1986), in which mate quality is an indirect cue used by the other parent to assess the quality of the offspring in each

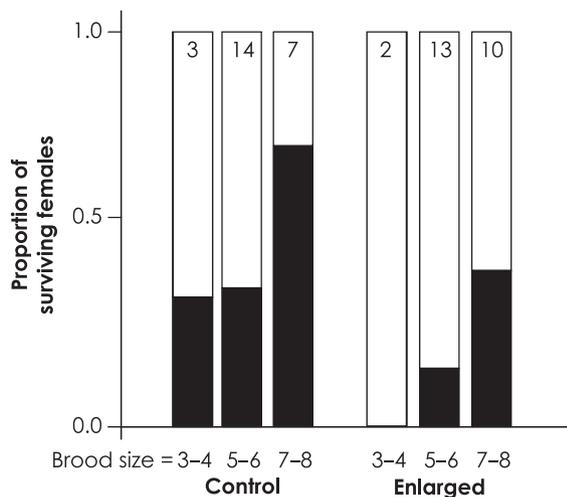


Fig. 3 Female survival rate in relation to brood treatment (control broods vs. broods with enlarged yellow patch) and to brood size (brood sizes have been pooled for graphical purposes). Black bars = survived females; open bars = not survived females (numbers in the bar represent the total number of females per brood size).

breeding episode and to adjust its parental allocation accordingly (see Sheldon, 2000 for a review). A strategic allocation of the overall parental care accorded to a given clutch seems particularly likely in altricial species with synchronous hatching, in which nestling quality can show a large variation not only within clutches but also among clutches. In our rock sparrow population, indeed, the size of the yellow breast patch varied more among clutches than within clutches. This variation among clutches may be partly because of seasonal effects and genetic/maternal effects, as patch size is correlated with laying date ($r_{44} = 0.29$, $P = 0.05$) and mother's patch size, although not significantly so ($r_{27} = 0.32$, $P = 0.10$). From this perspective, it would be interesting to measure the effect of manipulating the ornament of the whole brood on parental effort in those species in which the ornament manipulation of only one part of the brood did not elicit any change in parental care from the parents (e.g. great tit: Tschirren *et al.*, 2005).

As regards parents' effort in feeding the brood, only females responded to patch manipulation by increasing their nestling feeding rate, although male rock sparrows are capable of taking care of the whole brood if deserted (Pilastro *et al.*, 2001; this study). Nestling provision rate of nondeserted males is usually very low (on average less than 10% of the total feeding trips, Pilastro *et al.*, 2003), and most males stop helping to feed the nestlings one week after they have hatched. It is therefore not surprising that males did not respond to nestling manipulation by increasing their feeding rate, and so even if breast patch enlargement affected both male and female defence behaviour (more active in the enlarged group), there is evidence that nestling ornamentation influenced different aspects of parental allocation in the two sexes. The difference between male and female parental strategy may depend on the relative costs of nestling feeding and defence in terms of re-mating opportunities (higher for males) and future survival costs (females have on average lower survival rate than males, Tavecchia *et al.*, 2002). These results parallel those of previous studies investigating male parental allocation in relation to female ornamentation in this rock sparrow population (Griggio *et al.*, 2003; Pilastro *et al.*, 2003; Matessi *et al.*, 2009). In all these studies, female ornamentation affected male parental effort in nestling defence but not in nestling feeding rate.

When the first brood is perceived as being of a high quality, we may expect females to change their parental allocation strategy and increase their investment in the first brood, at the expense of a possible future second brood. About 20% of the females in this population are able, on average, to lay a second clutch in the same breeding season after a successful attempt (Pilastro *et al.*, 2001). The increased parental investment in the first brood should reduce the opportunities for the females to lay a second clutch within the same season. In contrast, we found a positive effect of treatment and first brood

laying date on the probability of laying a second clutch. Considering that carotenoid ornaments are likely to be condition-dependent and hence linked to environmental conditions, this result suggests that the quality of the current offspring may be used by the parents as a cue to assess environmental conditions during the current breeding season and consequently to adjust their subsequent reproductive allocation.

The analysis of local recruitment of the adults suggests that, in the enlarged group, females, but not males, suffered a higher post-reproductive mortality rate. The increased reproductive effort of the females of the enlarged group (increased feeding rate in the first brood and higher proportion of double-brooding) was therefore traded-off against future survival. The finding that males' survival was not affected by nestling ornament manipulation is not surprising, as males contributed very little to nestling feeding. Double brooding was also unlikely to have affected males' reproductive effort, as in 72.2% of the cases females changed mate between the first and the second broods. Our results indicate that large patch size reflects high offspring quality (this study and M. Griggio, unpublished results) and serves to the parents as a signal to indicate optimal (probably environmental) conditions for investment in present reproduction. Parents, and in particular mothers, might allocate all their resources to current parental care and to producing a second high-quality clutch in the same breeding season at the expense of future maternal survival.

We found no significant effect of ornament manipulation on offspring survival. This lack of effect may be because of insufficient statistical power, possibly further reduced by the pronounced difference in the offspring recruitment rate between years (which was not observed in the adults). Alternatively, considering that ornament manipulation occurred at a late nestling stage, it is possible that the increased parental care may be insufficient to translate into increased survival probability.

In conclusion, our study reveals that parents fed and defended more intensely the broods in which carotenoid-based plumage ornaments were enlarged. The females in the enlarged group were also more likely to lay a second clutch within the same breeding season, suggesting that the quality of the offspring produced during the first breeding attempt influences the reproductive allocation strategy of the whole breeding season.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results from the analyses in which feeding rate before breast patch size manipulation was entered as covariate.

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